

Spring 2014

# Pollution in Stone Town's Coastal Waters: An Assessment of Environmental Influences on Fecal Contamination

Socorro Lopez  
*SIT Study Abroad*

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# Pollution in Stone Town's Coastal Waters

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An Assessment of Environmental Influences on Fecal Contamination

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Spring 2014

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Ecology and Natural  
Resource Management

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## **Abstract**

Urbanized areas in Tanzania, including Zanzibar Town on Unguja, are struggling to deal with the large amount of waste generated by growing populations and increasing tourist industries. In 2010, the waters surrounding Stone Town, a subsection of Zanzibar Town, were found to be highly polluted by fecal waste. This study attempted to determine whether pollution has lessened or worsened in the past four years. Furthermore, environmental components of the coastal area, particularly tides, were tested in order to determine whether they had an impact on pollution in the waters. Using the membrane filtration method, mean enterococci were quantified in two areas of Stone Town, Africa House and the Port. Results suggest that the pollution at both sites has worsened and continues to pose a serious risk to public health. Furthermore, spring and neap tides had a significant impact on enterococci concentrations at Africa House, but how these environmental fluctuations influenced concentrations at the Port was not entirely clear. If measures are not taken in the future to improve the pollution in Stone Town's coastal waters, there could be serious consequences to the local economy and the community's health.

## **1.0 Introduction**

### *1.1 Coastal Pollution in Tanzania*

The diverse habitats that are found in Tanzania's coastal regions sustain non-living and living resources that are vital to the ecological integrity of these areas (Mohammed, 2002). In the past, these resources have significantly contributed to social and economic development amongst local communities by sustaining livelihoods that are dependent upon fisheries and other related activities (Mohammed, 2002). In recent years, coastal regions have continued to be vital to Tanzania's economy; coastal tourism and aquaculture have become extremely important sources of revenue for the country (Mohammed, 2002). However, as the economy has flourished, local populations and industrial activities along the coast have increased substantially. Consequently, immense pressure has been placed on the resources that are found in coastal areas and pollution has become a serious issue (Mohammed, 2002).

In developing countries, such as Tanzania, rapidly urbanizing areas with high population growths often suffer from insufficient infrastructure and uncontrolled urban development (Scholz, 2004). Without proper infrastructure, developing urban areas find it difficult to manage the large amounts of waste that their growing populations produce (Scholz, 2004). Although most of Tanzanian's coastal waters have proven to be in "pristine" condition, the waters surrounding Dar es Salaam, Tanga, Mtwara, and Zanzibar are considered to be highly polluted (Mohammed, 2002). The pollution found in waters near these urbanized areas can be largely attributed to untreated municipal, industrial and agricultural wastes that coastal habitats near human settlements are exposed to (Mohammed, 2002). These harmful wastes are found in the environment because untreated sewage is deposited directly into coastal waters from cities and

towns, an issue that has been caused and exacerbated by the fact that waste management systems in Tanzania are either non-existent or largely inadequate in many areas (Mohammed, 2002).

With a growing population, large tourism industry, and an annual urban growth rate of 4.5%, Zanzibar is no exception to the waste management predicament that the rest of the country is facing. As a whole, the island does not have a national plan to control waste and around 70% of the waste that is generated is randomly disposed of (Kalin & Skoog, 2012). In the case of

Zanzibar Town, one of the most urbanized and populated areas in Zanzibar, only Stone Town, a subsection of Zanzibar Town, is sewerred (Moynihan, 2012; Sharpley & Ussi, 2012). Stone Town accounts for about 25% of the municipality's jurisdiction (Moynihan, 2010).

Currently, 2,289 septic tanks and 27 sewage outfalls, the locations of which are shown in Figure 1, service the entire area (Moynihan, 2010).

Collectively, these outfalls have been estimated to discharge around 2,200 m<sup>3</sup> of liquid waste into the ocean on a



**Figure 1.** Locations of Stone Town's sewage outfalls (Moynihan, 2010)

daily basis (Moynihan, 2010). In an attempt to treat the sewage, the Biological Oxygen Demand (BOD) is reduced by 60% (Moynihan, 2012). However, this treatment method does not

substantially reduce harmful contaminants or pathogens that are often found in human waste (Moynihan, 2012).

### *1.2 Quantifying Fecal Pollution in Marine Water*

When attempting to determine whether a body of water is contaminated by anthropogenic waste, concentrations of Fecal Indicator Bacteria (FIB), including total coliform, fecal coliform, *Escherichia coli* and enterococci (ENT), are used as indices of water quality (EPA, 2002). These types of bacteria are often found in the excrement of warm-blooded animals and humans (EPA, 2002). As of 2009, enterococcus was the only FIB accepted by the World Health Organization (WHO) and the U.S. Environmental Protection Agency (EPA) as an index of fecal contamination in marine waters that are used for recreational purposes (Dwight, ND). When compared to other FIB, the enterococcus bacterium serves as the most reliable indicator of fecal contamination because it has the greatest salinity tolerance (Moynihan, 2012).

Previous studies have found a positive correlation between higher ENT levels and a greater risk of gastrointestinal illness (EPA, 2002; Kolling, 2012). When found in recreational water, ENT acts as an indicator of fecal pollution and alludes to the possibility of pathogens that cause intestinal diseases (Kolling, 2012). It is important to note that indicator bacteria are not necessarily enteric pathogens, but they are often found in the same waste that contains disease-causing microbes (Noble, 2003). When a coastal area is being tested for fecal contamination, the EPA recommends that short-term monitoring be dependent upon on Single Sample Maximum (SSM) values, while long-term monitoring should be based upon the geometric mean of five or more samples collected within 30 days (Moynihan, 2012). Marine waters are deemed safe for recreation purposes when SSM values and geometric means are found to be less than 35 cfu/100



mL and 104 cfu/100 mL, respectively (Moynihan, 2012). If ENT concentrations are found to exceed these thresholds, a high risk of gastrointestinal illness is present (Moynihan, 2012).

### *1.3 Sources of Enterococci Bacteria in Marine Waters*

Since the presence of ENT in marine recreational waters was discovered to be a threat to public health, several studies have attempted to determine the most common sources of ENT bacteria in coastal waters (Dwight, ND). The most frequent anthropogenic sources of ENT in the environment are sewage outfalls and urban runoff. Permitted discharges, leaking infrastructure, spills and illegal dumping are all ways that urban runoff can ultimately become polluted by human waste (Dwight, ND). However, the ENT bacteria that ends up in the ocean as a result of urban runoff is largely dependent on the amount of rainfall that occurs (Dwight, ND). When there are higher levels of precipitation, there is a greater chance pollutants and other fecal matter will be picked up by runoff and deposit into ocean, causing higher ENT concentrations (Dwight, 2002).

Several studies have also examined the possibility of natural sources of ENT bacteria in the environment, including tides, sand, birds, kelp, plants, groundwater and wetlands (Dwight, ND). If significant amounts of ENT were able to be introduced by natural processes, this would implicate the bacteria's ability to serve as an index of human contamination in marine waters (Dwight, ND). In other words, ENT concentrations could falsely indicate that an area is contaminated by human waste when it is actually being contaminated by natural sources (Dwight, ND). However, most of these studies were found to lack scientific grounding and, if ENT was introduced into the environment via natural sources, concentrations would not be high enough to have a significant impact on overall ENT levels (Dwight, ND).

#### *1.4 Physical Influences on Enterococci Concentrations in Marine Waters*

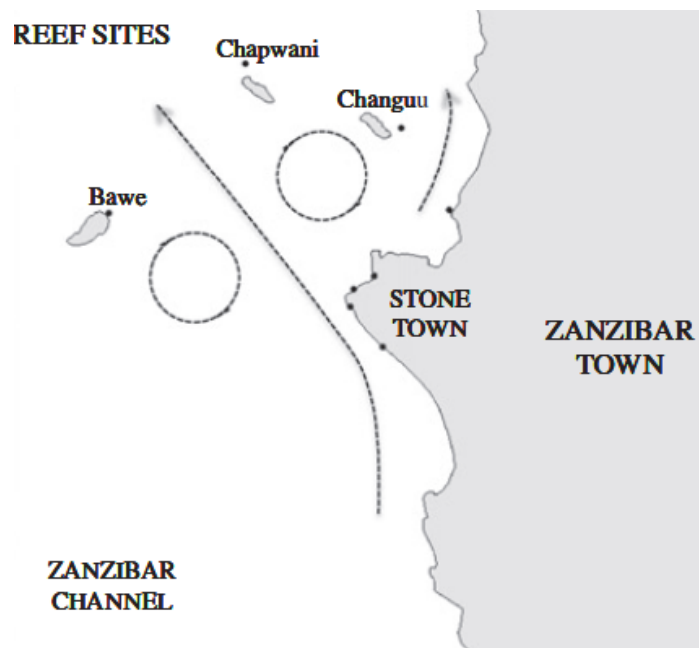
Once ENT bacteria have been deposited into a coastal environment by anthropogenic or natural sources, there are various factors that may have an impact on the bacteria's "fate and transport in marine waters" (Boehm, 2005). Rain, sunlight, tides, waves and temperature are all physical aspects that may influence FIB in marine waters (Boehm, 2005). However, aside from rain, the true impact of all of these factors on ENT is still largely unknown (Boehm, 2005). Nevertheless, studies have shown that ENT survival in the environment is largely dependent on the amount of sunlight that the bacteria receive (Kay, 2005). Transmission of sunlight in the water column is influenced by the turbidity, which is measured by total suspended solids (TSS) (Kay, 2005). This suggests that, if there was great deal of suspended solids in coastal waters, ENT bacteria would be less likely to survive.

Although it is an unlikely source of FIB in the environment, studies have illustrated that tidal forces have the potential to influence ENT once they have been deposited in marine waters (Boehm, 2005). In general, high tide lines are more likely to have a greater amount of bacteria, since higher tides cause the accumulation of almost all free floating objects (Dwight, ND). Nevertheless, a high tide does not necessarily correlate with a higher concentration of ENT, when compared to low tides. Studies have shown that ENT values during low tide regularly exceed high tide values (Dwight, ND). However, a study by Boehm in 2005 found that ENT levels were generally higher during the spring tide, when tidal range is at its maximum, as opposed to the neap tide, when tidal range is at its minimum (Boehm, 2005; NOAA, 2013). Furthermore, although there was a higher tendency of ENT to be present during the tidal ebb, when water is moving away from shore, as opposed to tidal flood, when water is moving towards shore, the results were not very consistent (Boehm, 2005). Overall, the true impact that tidal

forces have on amounts of ENT in marine waters is still largely unknown, and even if they do have an impact, there is great potential for concentrations of ENT to be simultaneously influenced by numerous other constituents.

### *1.5 Enterococci Bacteria in Stone Town's Coastal Waters*

A study conducted in 2010 by Moynihan found that the waters surrounding Stone Town had statistically significant levels of ENT bacteria, which exceeded limits that are deemed safe for swimming and other recreational activities by the EPA (Moynihan, 2010). Although Stone



**Figure 2.** The “great pass” in the Zanzibar Channel  
(Moynihan, 2012)

Town's sewage outfalls deposit waste 55 meters away from the shore, two eddies on either side of a strong current in the Zanzibar Channel known as the “great pass”, illustrated in Figure 2, prevent pollution from being distributed into deeper waters and becoming better mixed in the water column (Anderson, 1994). Another factor that was speculated to be a contributing factor to the high ENT concentrations in the area

was elevated precipitation rates caused by the SE monsoon (Moynihan, 2010). The SE monsoon typically causes a heavy rain season in Zanzibar during March and April (Moynihan, 2010). Elevated rainfall levels usually correlate with higher runoff, sewage outflow and, consequently, fecal indicator bacteria (Moynihan, 2010).

Due to Stone Town's inadequate waste management system, this study hypothesized that, four years after initial monitoring of fecal contamination in the area, water samples would still exhibit high amounts of ENT that would exceed EPA regulations surrounding safe recreational water standards. Furthermore, ENT concentrations were predicted to be higher during spring tide as opposed to neap tide. Since the tidal range of Zanzibar's semi-diurnal tides during spring and neap cycles is quite drastic, differences in the amount of ENT present were expected to vary to a large extent. However, high levels of TSS in samples were predicted to correlate with lower ENT counts. Finally, because this study was conducted in the midst of the SE monsoon, high precipitation rates were expected to have a direct relationship with higher ENT concentrations.

## 2.0 Study Area



Figure 3. (left) Tanzanian coastline (Dept. of Environment, ND)

Figure 4. (top right) Stone Town, Unguja (Google Maps, 2014)

Figure 5. (bottom right) Sample sites in Stone Town (Google Maps, 2014)

Located in the Indian Ocean about 32 kilometers off the coast of East Africa, Zanzibar consists of two main islands, Unguja and Pemba, depicted in Figure 3 (Sharpley & Ussi, 2012). The Zanzibar archipelago also includes approximately 50 smaller islets (Sharpley & Ussi, 2012). Since 1964, Zanzibar has been a semi-autonomous State of the United Republic of Tanzania (Sharpley & Ussi, 2012; Moynihan, 2012).

This study took place in Stone Town, shown in Figure 4. Stone Town is the oldest subsection of Zanzibar Town, Zanzibar's capital on Unguja (Moynihan, 2012). With a growing urban population and tourism industry, Zanzibar Town fits the criteria of an area that is affected by a coastal environment that is contaminated by human waste. Water samples were collected at two areas along Stone Town's waterfront: Africa House ( $6.165^{\circ}\text{S}$ ,  $39.187^{\circ}\text{E}$ ) and at the Port ( $6.158^{\circ}\text{S}$ ,  $39.192^{\circ}\text{E}$ ), the locations of which are illustrated in Figure 5. Both of these sites serve as hubs for a variety of anthropogenic activities including boat activity and swimming.

## Methodology

### *3.1 Sample Collection<sup>1</sup>*

Water samples were collected within half an hour of high and low tides during the spring and neap tides. Weather conditions were recorded on site. Before samples were collected, glass bottles were sterilized in an electric sterilizer at the Zanzibar Water Authority (ZAWA) laboratory. Upon arrival at each site, the bottles were rinsed with sample water three times and then collected in four separate containers six inches below the surface. While collecting a sample, the container was pointed towards the flow of incoming water in order to avoid contamination.

On site, temperature for all samples was measured using a thermometer and pH was measured for the first two samples at each site using pH paper. The pH for the remaining samples was measured at the ZAWA lab using a hand held pH meter in order to obtain a more accurate reading. Once collected, the samples were transported on ice to the Institute of Marine Science (IMS), where one container was frozen for later analysis of total suspended solids and salinity levels. The remaining samples were transported to the ZAWA lab in order to perform microbiological analysis within six hours of initial collection time.

### *3.2 Enterococci Membrane Filtration Procedure*

In order to quantify fecal contamination in Stone Town's coastal waters, the membrane filtration (MF) procedure<sup>2</sup> outlined by the US EPA's Method 1600 was utilized as a guideline for the detection and enumeration of ENT bacteria in water samples. Prior to carrying out the membrane filtration procedure, mE agar, a selective medium that promotes the growth of only

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<sup>1</sup> See appendix for water quality sheet used to record data

<sup>2</sup> See appendix for more details concerning MF procedure

enterococci bacteria, was prepared (HiMedia Laboratories, ND). The agar was prepared in a ratio of 42 grams of agar powder to 1000 mL of distilled water and boiled to dissolve any suspended particles. Four to five millimeters of agar was then poured into sterilized petri dishes. After solidifying, agar plates were labeled and placed in the refrigerator for later use.

Once at ZAWA, all equipment was sterilized with ethanol or distilled water. In preparation for membrane filtration, a sterilized membrane filter was placed in the filtration apparatus in order to detain any bacteria in the water sample. For each sample, water from each site was diluted with distilled water by half, 1/8 or 1/16. Next, 100 mL of each diluted sample was filtered through the membrane. The membrane was then removed with sterilized forceps and placed on a previously prepared agar plate. Two to six replicates were performed for each water sample. After the agar plates had been prepared, the petri dishes were inverted and incubated at  $35\pm 2^{\circ}\text{C}$  for 48 hours. Once the incubation period was over, light and dark red colonies were enumerated as ENT. The data recorded was converted to colony forming units (CFU)/100 mL using the equation  $CFU/100\text{ mL} = (\text{original colony count/mL of sample filtered}) * 100$  (EPA, 2002).

### *3.3 Total Suspended Solids & Salinity Levels*

Aside from microbiological analysis, salinity and total suspended solids were also tested for each sample. The EPA Method 160-2 served a guideline for determining TSS, while salinity readings were conducted under the supervision of the lab bench at IMS. Twelve hours before analysis, samples were removed from the freezer and allowed to thaw. Once samples were at room temperature, a refractometer was used to determine the salinity percentage for each water sample.

After salinity measurements were recorded, the rest of the sample was analyzed for TSS via the MF technique. Prior to filtering, the weight of each glass fiber filter paper was recorded and labeled. Then, for each sample, a new filter paper was placed in the filtration apparatus, samples were shaken well, and 250 mL of the sample were filtered through the membrane. Filter papers were then placed in an oven overnight to eliminate any remaining water. The next day, filter papers were weighed again, and the original weight was subtracted from the final weight to determine the weight of total suspended solids. The data recorded was converted to TSS in mg/L using the equation  $TSS \text{ in mg/L} = (\text{weight of suspended solids} * 1000) / \text{liters of sample filtered}$  (EPA, 1971).

### *3.4 Rainfall Data*

An interview was conducted with Mr. Mohammed Ngwali, the Head of the Zanzibar Meteorological Department, in order to gather data pertaining to Stone Town's daily precipitation rates.

### *3.5 Challenges in Data Collection*

Along with EC, fecal coliform and total coliform were intended to be quantified with the purpose of comparing various bacteria concentrations present in water samples. Unfortunately, due to an inability to obtain total coliform agar, this type of bacteria analysis was excluded from the study. Furthermore, in relation to ENT detection, there was not sufficient time to perform serial dilutions for each sample and the most probable number (MPN) was not determined. For several samples, colony counts exceeded 200, and therefore, the data may be statistically inaccurate according to US EPA standards (EPA, 2002). Also, mean ENT data for cultures that had more than 200 colonies may be lower than the true values because the large numbers of ENT



colonies may have inhibited growth due to inadequate space for the bacteria to flourish. Furthermore, the ENT data for the third set of samples may be skewed since the cultures were not incubated for the full 48 hours; a power outage occurred during the incubation period for approximately 1.5 hours. Also, certain data from the second and fifth set of samples were not included in the results because an unidentifiable bacterium was growing in the petri dishes with the ENT bacteria and, therefore, the sample was considered to be contaminated. Finally, it is important to note, that the EPA Method 1600 discusses the use of mEI agar, a medium that has proven to be more selective and precise than other agars.<sup>3</sup> Although it is less precise in promoting ENT growth, mE agar was used in this study because mEI agar was not available.<sup>4</sup>

In regards to difficulties associated with the other tests performed, the pH readings for the first two sets of samples may be inaccurate since the pH paper was very difficult to read. Furthermore, when testing samples for TSS, the filters were not rinsed with deionized or distilled water after samples were filtered. Rinsing is often recommended in order to dissolve any salt that may have been deposited on the filters and add to the filter's final weight. Therefore, the final values recorded for TSS may be higher than the true values when taking into account the possible weight of salt.

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<sup>3</sup> See appendix for more details concerning the directions for and use of mE agar

<sup>4</sup> See appendix for more details concerning the specificities of mE and mEI agars

## 4.0 Results

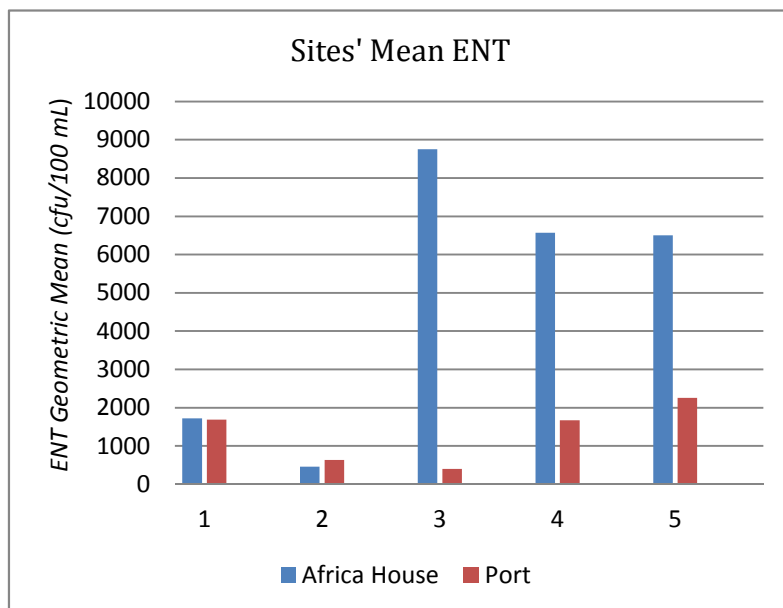
### 4.1 Stone Town's Water Quality

**Table 1.** Stone Town Water Quality Data

Site	Sample #	Salinity (%)	pH	Temp (°C)	Percip. (mm)	Mean ENT (cfu/100 mL)
Africa House	1	33	8.0±1	30	0.0	1720
	2	33	8.0±1	31.5	0.0	460
	3	25	8.5	31.5	4.8	8748*
	4	27	8.4	29.5	2.0	6568
	5	28	8.6	29	2.0	6503
Port	1	25	8.0	30.5	0.0	1685
	2	30	8.0	32	0.0	638
	3	26	8.6	30	4.8	399*
	4	33	8.5	30	2.0	1668
	5	29	8.6	28	2.0	2253

\*Samples were not incubated for entire 48 hours due to 1.5 hour power outage

Table 1 illustrates the data that was collected from the sites in Stone Town. The mean ENT concentration for all of the samples at both sites exceeded the 104 cfu/100 mL threshold set by the EPA. The sample that had the lowest ENT count, the second sample taken at Africa



**Graph 1.** Mean ENT at two sites in Stone Town

House, was still four times higher than the EPA's recreational water quality standards. As illustrated in Graph 1, samples from Africa House, except for sample 2, had higher colony counts than those from the Port. The arithmetic mean of all five samples from

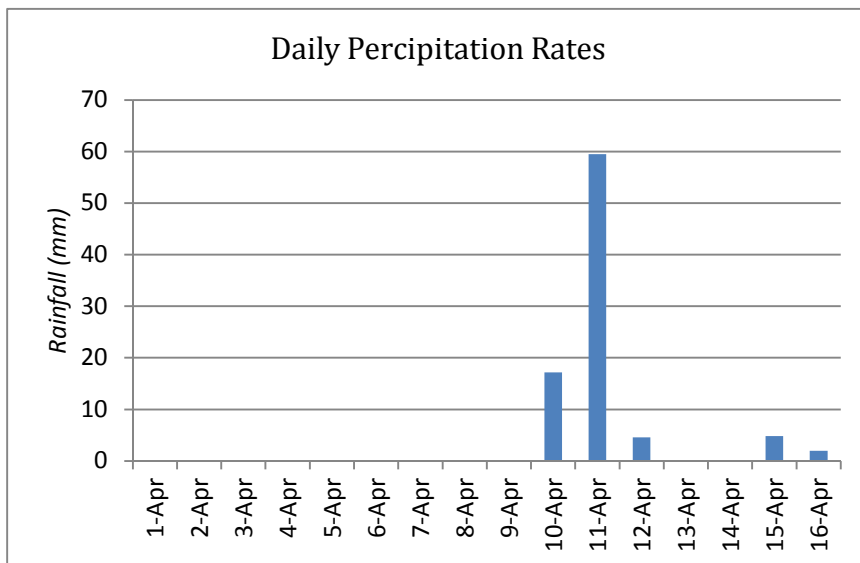
Africa House, 4799 cfu/100mL, was much higher than the average of the Port's five samples, 1328 cfu/100 mL.

#### 4.2 Impact of Rainfall and TSS on Enterococci Concentrations

Table 2. April 2014 rainfall data (Zanzibar Meteorological Department, 2014)

Day	Rainfall (mm)
4/1	0.0
4/2	0.0
4/3	0.0
4/4	0.0
4/5	0.0
4/6	0.0
4/7	0.0
<b>4/8</b>	<b>0.0</b>
<b>4/9</b>	<b>0.0</b>
4/10	17.2
4/11	59.5
4/12	4.6
4/13	0.0
4/14	0.0
<b>4/15</b>	<b>4.8</b>
<b>4/16</b>	<b>2.0</b>

*Note: sampling dates bold and italicized*



Graph 2. April 2014 rainfall data (Zanzibar Meteorological Dept, 2014)

As shown in Table 2 & Graph 2, there was very little rainfall during the days that samples were collected. However, 2 mm of rainfall fell when the fourth and fifth samples were being collected, which could have caused spring tide ENT counts to increase. Furthermore, the highest rainfall recorded, 4.8 mm, occurred when the highest ENT count, 8748 colonies, was recorded at Africa House. However, on the same day, the lowest ENT count was also recorded at the Port, with 399 colonies. This suggests that the small amount of rainfall that occurred during the days when water was sampled did not significantly influence ENT counts. In order to prove that rain was not a defining factor in this study, a regression<sup>4</sup> was

<sup>5</sup> Regression was performed on pH, rainfall, temperature, and salinity data; the p value was greater than .05 for all variables and all variables were, therefore, considered to have statistically insignificant impacts on ENT geometric means

run on the data, which established that the degree to which rainfall data accounted for variation in ENT concentrations was statistically insignificant.

**Table 3.** TSS data

Site	Sam ple #	TSS (mg/L)	Mean ENT (cfu/100 mL)
Africa House	1	105.6	1720
	2	108.4	460
	3	93.2	8748
	4	154.8	6568
	5	211.2	6503
Port	1	71.6	1685
	2	94.8	638
	3	100.8	399
	4	330	1668
	5	88	2253

Table 3 illustrates the total suspended solids data recorded for each sample. Although TSS was expected to have an inverse relationship with ENT colony counts, the highest TSS was recorded during the fourth sample at the Port, which had an ENT geometric mean that was not amongst the lower values recorded. Similarly, the lowest TSS value did not correlate with a higher ENT count. As with rainfall, a regression was used to determine the degree to which TSS data accounted for changes in ENT geometric means and the relationship between the two

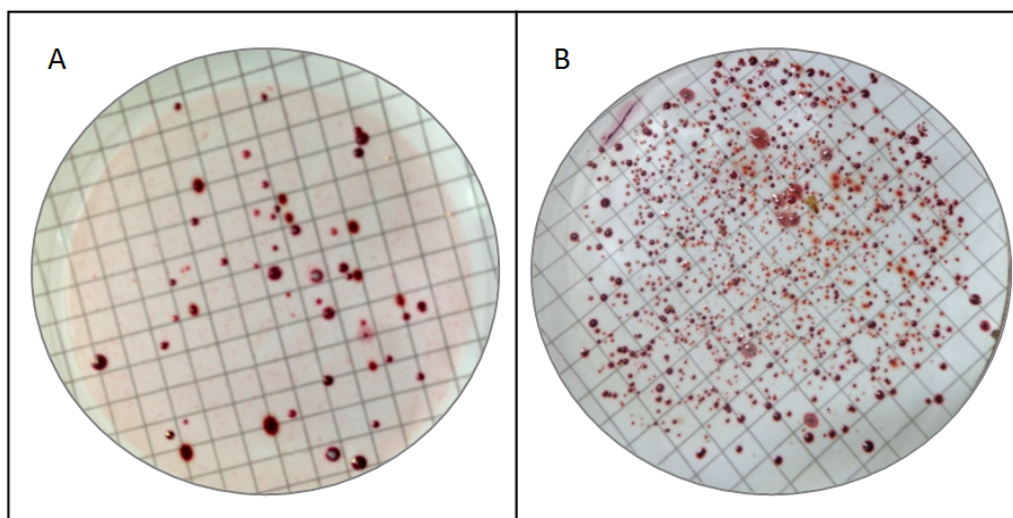
variables was found to be statistically insignificant.

#### *4.3 Impact of Spring and Neap Tides on Enterococci Concentrations*

**Table 4.** Tidal and ENT data

Site	Sample #	Tidal Range	Type of Tide	Meters (above sea level)	Mean ENT (cfu/100 mL)
Africa House	1	Neap	Low	1.74	1720
	2	Neap	High	2.49	460
	3	Spring	High	3.97	8748
	4	Spring	Low	0.27	6568
	5	Spring	High	4.07	6503
Port	1	Neap	Low	1.74	1685
	2	Neap	High	2.49	638
	3	Spring	High	3.97	399
	4	Spring	Low	0.27	1668
	5	Spring	High	4.07	2253

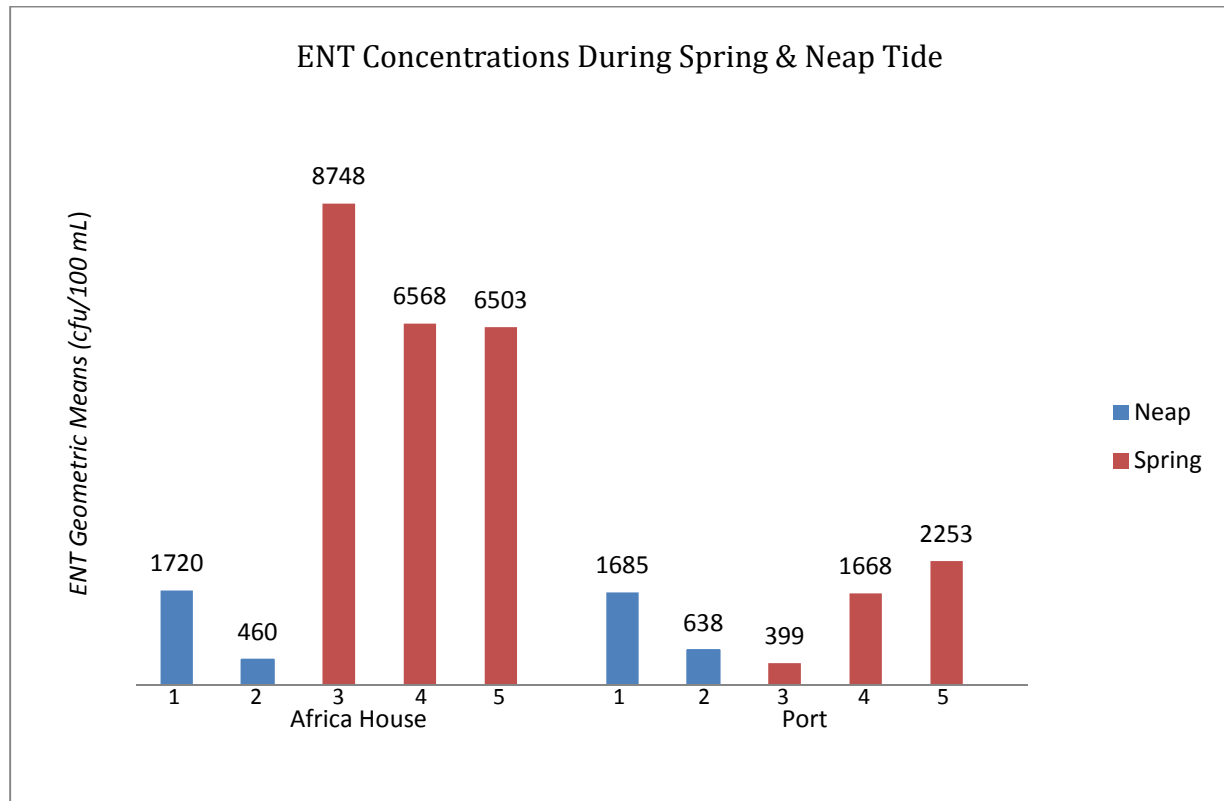
Data surrounding tidal characteristics on the days that water samples were collected are shown in Table 4. In terms of Africa House, ENT concentrations during spring tide were all significantly higher than concentrations during neap tide. As shown in Figure 6, there was a stark difference between sample 2 (A), when the lowest number of colonies were counted at Africa House, and sample 3 (B), when the highest number of colonies were recorded. Sample 2 and 3 were collected during neap and spring tides, respectively. Between these two samples, there was a 19-fold increase in the number of colonies seen.



**Figure 6.** ENT colonies present during neap tide (A) and spring tide (B)

The data collected from the Port, however, is a little less straightforward. The lowest recording of ENT data, 399 colonies, was during spring tide. Nevertheless, the highest ENT count, 2253 colonies, was also recorded during spring tide. Furthermore, samples 1 and 4, which were collected during neap and spring tide, respectively, were found to have very similar ENT geometric means of 1685 and 1668 colonies. On the other hand, sample 2, which was collected during neap tide, generated 638 colonies, while sample 5, which was collected during spring tide, produced 2253 colonies. As opposed to the other three samples, samples 2 and 5 exhibited the

expected relationship between spring tide and elevated ENT concentrations, a pattern that was very apparent at Africa House, as illustrated in Graph 3.



**Graph 3.** ENT Concentrations during spring and neap tides

## 5.0 Discussion

### 5.1 Changes in Stone Town's Water Quality

In 2010, ENT concentrations at Africa House and the Port were found to greatly exceeded recreational swimming standards. Both of the sites were sampled on the same day when there was a total of 35.2 mm of rainfall. Africa House was found to have a mean ENT count of  $377.5 \pm 29.9$  cfu/100mL, while the Port's mean ENT count was determined to be  $1716 \pm 427.1$  cfu/100 mL, although the data was originally deemed as “too numerous to count” (Moynihan, 2010; Moynihan 2012). It was noted that ENT counts could have been higher for the Port based on the observation that the colonies did not have enough room to grow, a factor that could have significantly hindered the true growth rate (Moynihan, 2010). When compared to the samples collected in 2010, all of the Africa House samples' ENT counts exceeded the initial ENT concentration of  $377.5 \pm 29.9$  cfu/100mL. On the other hand, only two of the five samples collected at the Port surpassed the 2010 ENT concentrations of  $1716 \pm 427.1$  cfu/100 mL,

The fact that all of the samples' ENT means greatly exceeded 104 cfu/100 mL clearly demonstrates that Stone Town's waters are highly polluted and should not be used for recreational purposes. Furthermore, Africa House's increased ENT concentrations illustrates that Stone Town's coastal contamination is worsening in certain areas and little has been done to improve water quality since 2010. The increase in concentrations near Africa House is particularly alarming because of its location in the southern part of Stone Town. Since currents in this area cause waters to flow northward to the “great pass”, waters are believed to have better circulation than areas in the northern part of town, especially near the Zanzibar Port (Moynihan, 2010). When taking into account ocean circulation patterns, ENT concentrations at Africa House should theoretically be lower than those at the Port where circulation is believed to be poorer.

However, the data shows that the exact opposite situation is occurring. This suggests that different factors in the environment have had an influence on changes in fecal contamination at different locations.

One possible explanation for the increase in ENT values at Africa House could be that a greater quantity of sewage is currently being discharged into the ocean in comparison to four years ago. This may be due to a steadily growing population in the area or an influx of tourists visiting the island. Zanzibar's urban population is expected to grow to 470,000 by 2015, a large increase from the estimated population of 206,292 in 2002 (Moynihan, 2012). Also, according to the Stone Town Conservation and Development Authority, approximately 95% of the 90,000 tourists that visit Zanzibar each year pass through Stone Town at some point (STCDA, ND). Another explanation of the elevated ENT rates at Africa House could be that the degradation of the sewage system in Zanzibar is allowing greater amounts of sewage to be discharged closer to shore via leaks or other damages. Zanzibar's sewage system was installed in the 1920's and, aside from being inadequate, the sewer systems that are in place in Tanzania are considered to be in "an advanced state of disrepair" (Mohammed, 2002).

In terms of the ENT values recorded for the Port, changes in pollution levels are unclear. In comparison to the amount of ENT that was recorded in 2010, two samples produced significantly lower ENT counts, two samples saw similar, yet slightly lower, ENT concentrations, and one sample was significantly higher than the original count. Overall, the Port's average ENT count, 1328 cfu/100 mL, was slightly lower than the initial count of  $1716 \pm 427.1$  cfu/100 mL. This suggests that pollution levels have slightly decreased in the area. However, when other factors are taken into account, there are several explanations for the



minimal changes seen in ENT concentrations at the Port from current and past monitoring results.

When comparing current ENT concentrations at the Port with those recorded four years ago, it is important to take into account the difference in rainfall data between the two studies. The samples in 2010 were collected on a day when there was a high amount of rainfall, an environmental aspect that is linked to higher FIB concentrations, while the most recent samples were collected when very little rainfall occurred. If greater precipitation rates had been experienced when samples were collected, ENT counts would have probably been higher and changes in the site's water quality would have been more apparent. Relatively stable pollution levels at the Port could also be attributed to ocean circulation patterns that may be preventing ENT from areas with higher contamination, such as Africa House, from being distributed to the northern part of town where water circulation is known to be poor.

### *5.2 Environmental Impacts on Enterococci Concentrations*

Aside from illustrating that Stone Town's marine waters are extremely polluted by fecal contamination, the results also revealed that waste is not homogeneously dispersed in Stone Town's waters even though sewage outfalls are situated along the coast in a uniform fashion. This trend was also observed in Moynihan's study in 2010. Since the ocean is an incredibly dynamic environment, there are numerous aspects that have the ability to influence the fate and transport of ENT in marine waters and ultimately determine levels of contamination along the coast. The low impacts of pH, temperature, salinity, rainfall and suspended solids had on ENT concentrations during the study provided for a better demonstration of how other factors in the environment, such as tides, are influencing the distribution of waste in Stone Town's waters.

Based on the study's results, there was no relationship between high and low tides and increased or decreased ENT values. However, assuming factors that were not tested in this study are not influencing the distribution of ENT in the coastal waters, there was a correlation between spring tides and higher ENT counts at Africa House. Spring tides are thought to cause higher contamination rates because water reaches high water lines and allows for a "hydrological connection" between sources of fecal waste and the ocean (Boehm, 2005). Furthermore, "the higher and lower than average tides that accompany spring tides mobilize and allow flushing of, respectively, pollutants to the sea, or provide enhanced conditions for ENT persistence in seawater" (Boehm, 2005). During neap tides, Africa House exhibited significantly lower ENT counts, which are comparable with the levels of contamination observed at the Port throughout the study. Furthermore, during neap tide, there was no clear pattern as to which site had higher contamination.

Seeing as the impact of tidal range on ENT concentrations was not very clear at the Port, ENT concentrations at this site may be influenced by other environmental factors that were not tested in this study. However, if the third samples from both sites, the growth rates of which may have been influenced by the power outage, were omitted from the data, the impact of spring and neap tides on ENT concentrations becomes more apparent. Except for the sample taken at the Port during the neap tide's low tide, all ENT concentrations during spring tide surpass the concentrations recorded during neap tide.

### *5.3 Implications of Failing to Address Water Quality Issues*

There are several implications associated with poor water quality if no action is taken to improve water quality in Stone Town. One of the most obvious issues is the risk to public health

that the problem poses. If ENT values in recreational waters are higher than 104 cfu/100 mL, anyone who comes into contact with the water is at risk for skin rashes, nausea, vomiting, diarrhea and gastrointestinal illness (Haile, 1999). At both sites, people were observed to be swimming in the water and various other anthropogenic activities were occurring, including boat activity.

One of the simplest and most achievable solutions for this problem is education. In order to gauge the public's awareness of water quality issues, the Moynihan's study interviewed locals and tourists and found that there was an overall lack of knowledge surrounding the topic (Moynihan, 2010). Furthermore, a majority of interviewees swam in Stone Town regularly, even if they knew that the water was polluted (Moynihan, 2010). People who are likely to come into contact with the water, especially vulnerable groups such as children and tourists whose immune systems may be more susceptible to enteric pathogens in the water, should be informed about potential health risks and situations when these risks are exacerbated by environmental factors. This can be achieved through informational pamphlets and seminars, signs near popular swimming destinations in town, community forums and other methods that effectively circulate vital information that is currently, widely unknown.

Aside from health issues, a failure to address highly polluted coastal waters will result in devastating economic and ecological consequences. Several studies have been performed on elevated nutrient levels in Stone Town's coastal waters, which are associated with large amounts of partially treated sewage in the marine ecosystem. A study that monitored eutrophication in Stone Town for an entire year, found that nutrient levels exceeded conditions that permit corals to grow and thrive (Mohammed & Magaya, 1997). Eutrophication is known to cause large algal blooms that prevent other organisms such as fish from having access to oxygen (Mohammed &

Magaya, 1997). Subsequently, vital species begin to disappear and large ecosystems, such as coral reefs, eventually collapse. Important economic activities in Unguja, including fishing, aquaculture and tourism, will suffer tremendously if the vitalities of surrounding coral reefs are compromised. In order to begin addressing the issues associated with coastal pollution, however, the true extent of the issue needs to be determined.

In order to gain a clearer understanding of how sewage is being distributed in coastal waters in Zanzibar, FIB need to be monitored more regularly and extensively. Currently, there is no other data surrounding ENT concentrations in Stone Town's coastal waters, or in any other urbanized area on Unguja, aside from Moynihan's study in 2010 and this study, both of which were conducted during the SE monsoon and for very short periods of time. Greater knowledge of how ENT concentrations are changing on a daily, or evenly monthly, basis would provide data that would improve our understanding of the extent of pollution and fecal contamination in Stone Town's marine waters. Once the scope of information surrounding pollution in Stone Town is broadened, integrated mitigation strategies that produce concrete results can be established in order to curb the detrimental impacts that poor water quality has on the surrounding environment.

Measures that can be taken in order to improve coastal pollution in Stone Town include fixing damaged components of the sewage systems, treating sewage before it is released into the environment, and ensuring that drainage systems help to decrease the amount of fecal matter that is deposited into marine water via urban runoff. However, when solutions are being implemented, it is necessary to take into account environmental influences on the distribution of waste in coastal waters. Waste containing harmful pathogens should try to be prevented from being released into the environment when ENT concentrations are known to be highest near

shore, including during high rainfall and spring tides. Furthermore, the sewage outfalls that dispose of waste should be extended in order to ensure that sewage is able to reach deeper waters. Although the most effective solutions to decreasing pollution in Stone Town are likely to be incredibly costly, taking meaningful action is essential to ensuring that the well being of the coastal environment and the people that depend on its resources are secured in the future.

## 6.0 Conclusion

Preserving the integrity of Tanzania's coastal regions is vital to the well-being of the natural resources that are found in these areas, as well as the people that are dependent on these resources. However, due to increased populations and inadequate infrastructure, urban areas are experiencing severe issues with pollution and coastal areas are becoming extremely contaminated by fecal waste. Based on elevated concentrations of ENT bacteria in water samples, the study found that water quality continues to be unfit for recreational activities along the Stone Town waterfront even though it is used for such activities on a daily basis. In the past four years, ENT concentrations have substantially worsened in the waters around Africa House, suggesting that the area has become more polluted over time. Furthermore, ENT concentrations were found to be higher during the spring tide at this area. On the other hand, ENT concentrations at the Port did not correlate as strongly with tidal forces and there was a slight decrease in pollution over time. Since pH, rainfall, temperature, salinity and TSS did not have a significant impact on ENT concentrations, other environmental variables that were not examined during this study are believed to have influenced the distribution of waste in the coastal waters near the Port. In order to determine what is influencing bacteria concentration in Stone Town's waters, more research needs to be done on ENT concentrations and the environmental variables that have the ability to influence waste in marine waters. Once a greater understanding of these issues is attained, effective measures and integrated mitigation strategies should be implemented in order to prevent detrimental impacts on the economy and coastal environment.

## **7.0 Recommendations**

Overall, a great deal of knowledge is still needed concerning the numerous biological and physical factors that influence the transmission of ENT in coastal waters. In the future, studies should continue monitoring the levels of contamination in Stone Town, as well as other urbanized areas in Zanzibar. Furthermore, ENT should be enumerated during the northeast monsoon period in order to determine how ENT values are influenced by climatic changes. This particular study should be replicated with the intent of gaining a better understanding of how ENT concentrations fluctuate in response to spring and neap tides. It would also be beneficial to test ENT levels during tidal ebb and flood in order to determine why spring tides are associated with higher ENT counts. Finally, research should be done to establish the most effective way to educate the public and tourists about water quality and fecal contamination in order to begin the process of implementing solutions for this extremely complicated issue.

## 8.0 References

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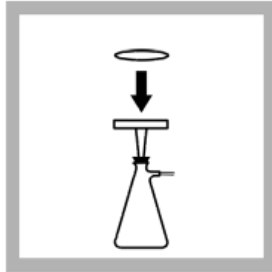
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## Appendix

### 1. Data Collection Sample Sheet (EPA, 2009)

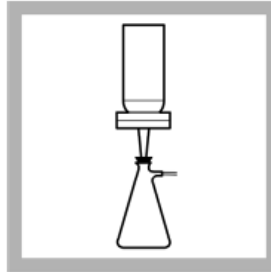
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## 2. Membrane Filtration Technique (EPA, 2009)



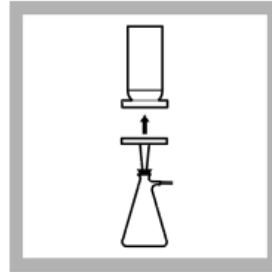
1. Set up the Membrane Filter Assembly. Using sterilized forceps, place a membrane filter, grid side up, into the assembly.

To sterilize forceps, dip forceps into alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.

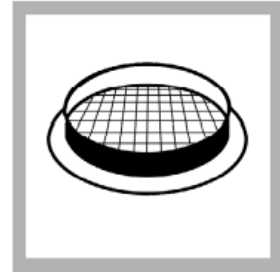


2. Prepare the necessary dilutions to obtain the proper sample size. Invert the sample for 30 seconds to mix. Pour sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls with 20 to 30 mL of sterile buffered dilution water. Apply vacuum. Rinse again two more times.

Release the vacuum when the filter is dry to prevent damage to the filter.



3. Turn off the vacuum and lift off the funnel top. Using sterilized forceps, transfer the membrane filter to the previously prepared agar plate.



4. With a slight rolling motion, place the filter, grid side up, on the prepared m-EI agar plate. Check for air trapped under the filter and make sure the entire filter touches the agar.

Replace the petri dish lid.

### 3. Differences in Specificities Between mE Agar and mEI Agar (UNC, ND)

“The term enterococci is used by some as synonymous with the term fecal streptococci. Indeed, the taxonomy of the "fecal streptococci" is now based on the name "enterococci". In the U.S.A. the term enterococci as used by the U.S. Environmental Protection Agency is meant to include only *E. faecalis* and *E. faecium* and to exclude *S. bovis*, *S. equinus* and the Group Q streptococci. A medium was developed that was intended to specifically detect the enterococci by a membrane filter method (Levine et al., 1975). The advantage of this test is that it excludes some streptococci that are more likely to be associated with non-human fecal contamination, and hence is considered a more human-specific fecal indicator. However, this contention is somewhat flawed because some of the excluded streptococci, such as *S. bovis*, have been found in human feces. Furthermore, the test for enterococci does not exclude some subspecies of *E. faecalis* (e.g., *liquefaciens*) that are not always associated with fecal contamination, and it detects species of enterococci are also found naturally in the environment, such as *E. mundtii* and *E. casseliflavus*. Hence, it is not strictly a feces-specific medium.

Epidemiological and microbiological studies in the U.S.A. demonstrated that concentrations of enterococci in swimming waters correlated with risks of gastrointestinal illness in swimmers (Cabelli et al., 1982; 1983). The results of these studies led the U.S. EPA to recommend enterococci as the bacterial criterion for the acceptability of swimming waters (U.S. EPA, 1985). More recent studies in the UK and elsewhere led to the World Health Organization recommending the use of “intestinal enterococci” as the preferred indicator of fecal contamination of recreational waters. Intestinal enterococci are defined as a subgroup of the fecal streptococci that can grow at 44°C, hydrolyze MUG in the presence of thallium acetate, nalidixic acid and 2,3,5-triphenyltetrazolium chloride in specified liquid media.

The development of new membrane filter media, the best of which is now called mEI medium, greatly improved the ability to detect primarily the enterococci. In the U.S these organisms are defined as the true "enterococci. However, mEI does not detect only *E. faecalis* and *E. faecium*, because as previously noted, some fecal streptococci will grow on mEI medium. However, this may not be a major deficiency because other species of enterococci have been detected in human as well as animal feces.” (UNC, ND)

#### 4. *mE Agar Preparation (HiMedia Labs, ND)*

M-Enterococcus Agar Base is a selective medium used in membrane filtration procedures as well as a direct plating medium, for isolation and enumeration of Enterococci in water, sewage, food or other materials.

##### **Composition\*\***

<b>Ingredients</b>	<b>Gms / Litre</b>
Casein enzymic hydrolysate	15.000
Papaic digest of soyabean meal	5.000
Yeast extract	5.000
Dextrose	2.000
Dipotassium phosphate	4.000
Sodium azide	0.400
2,3,5-Triphenyl tetrazolium chloride	0.100
Agar	10.000
Final pH ( at 25°C)	7.2±0.2

\*\*Formula adjusted, standardized to suit performance parameters

##### **Directions**

Suspend 41.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT OVERHEAT OR AUTOCLAVE. Add 0.5 ml polysorbate 80 and 2 ml of 10% aqueous solution of sodium carbonate, if desired. Dispense into Petri plates.

Warning : Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.

## 5. Enterococci, TSS & Rainfall Raw Data

Sample	Sample Name	Date	mL Sample Filtered	Original Count	CFU/100 mL	Notes	GEO Mean	Temp	pH
1	AH1-L-EC1	8-Apr	50	824	1648			30	8
	AH1-L-EC2	8-Apr	50	894	1788			30	8
	AH1-L-EC3	8-Apr	50	863	1726		<b>1720</b>	30	8
2	M1-L-EC1	8-Apr	50	747	1494			30.5	8
	M1-L-EC2	8-Apr	50	1077	2154			30.5	8
	M1-L-EC3	8-Apr	50	743	1486		<b>1685</b>	30.5	8
3	AH2-H-EC1-1	9-Apr	12.5	56	448			31.5	8
	AH2-H-EC1-2	9-Apr	6.25	28	448	C		31.5	8
	AH2-H-EC2-1	9-Apr	12.5	59	472			31.5	8
	AH2-H-EC2-2	9-Apr	6.25	30	480	C		31.5	8
	AH2-H-EC3-1	9-Apr	12.5	76	608	C		31.5	8
	AH2-H-EC3-2	9-Apr	6.25	39	624	C	<b>460</b>	31.5	8
4	M2-H-EC1-1	9-Apr	12.5	91	728			32	8
	M2-H-EC1-2	9-Apr	6.25	44	704	C		32	8
	M2-H-EC2-1	9-Apr	12.5	70	560			32	8
	M2-H-EC2-2	9-Apr	6.25	37	592	C		32	8
	M2-H-EC3-1	9-Apr	12.5	52	416	C		32	8
	M2-H-EC3-2	9-Apr	6.25	30	480	C	<b>638</b>	32	8
5	AH3-H-EC1	15-Apr	12.5	1142	9136	PO: 1.5 h		31.5	8.5
	AH3-H-EC2	15-Apr	12.5	1000	8000	PO: 1.5 h		31.5	8.5
	AH3-H-EC3	15-Apr	12.5	1145	9160	PO: 1.5 h	<b>8748</b>	31.5	8.4
	M3-H-EC1	15-Apr	12.5	62	496	PO: 1.5 h		30	8.6
6	M3-H-EC2	15-Apr	12.5	29	232	PO: 1.5 h		30	8.6
	M3-H-EC3	16-Apr	12.5	69	552	PO: 1.5 h	<b>399</b>	30	8.6
7	AH4-L-EC1	16-Apr	6.25	499	7984			29.5	8.5
	AH4-L-EC2	16-Apr	6.25	409	6544			29.5	8.4
	AH4-L-EC3	16-Apr	6.25	339	5424		<b>6568</b>	29.5	8.4
8	M4-L-EC1	16-Apr	6.25	144	2304			30	8.5
	M4-L-EC2	16-Apr	6.25	114	1824			30	8.5
	M4-L-EC3	16-Apr	6.25	69	1104		<b>1668</b>	30	8.5
9	AH5-H-EC1	16-Apr	6.25			C		29	8.6
	AH5-H-EC2	16-Apr	6.25	414	6624			29	8.6

	AH5-H-EC3	16-Apr	6.25	399	6384	<b>6503</b>	29	8.6
	M5-H-EC1	16-Apr	6.25	474	7584		28	8.6
10	M5-H-EC2	16-Apr	6.25	71	1136		28	8.6
	M5-H-EC3	16-Apr	6.25	83	1328	<b>2253</b>	28	8.6

\*C = contaminated

\*P.O. = power outage

$Cfu/100\text{ mL} = (\text{original colony count/mL of sample filtered}) * 100$

### *Total Suspended Solids Data*

Sample	Sample Name	Weight of FP	Weight After	Suspended Solids	TSS in mg/L
1	AH1-L-S	0.2476	0.274	0.0264	105.6
2	M1-L-S	0.2488	0.2667	0.0179	71.6
3	AH2-H-S	0.2467	0.2738	0.0271	108.4
4	M2-H-S	0.2473	0.271	0.0237	94.8
5	AH3-H-S	0.2463	0.2696	0.0233	93.2
6	M3-H-S	0.251	0.2762	0.0252	100.8
7	AH4-L-S	0.2475	0.2862	0.0387	154.8
8	M4-L-S	0.2498	0.3323	0.0825	330.0
9	AH5-H-S	0.2462	0.299	0.0528	211.2
10	M5-H-S	0.2482	0.2702	0.022	88.0

$TSS\text{ in mg/L} = (\text{weight of suspended solids} * 1000) / \text{liters of sample filtered}$

*Daily rainfall as observed at Zanzibar Meteorological station for the month of April 2014*

Day:	Rainfall (mm):
1	0.0
2	0.0
3	0.0
4	0.0
5	0.0
6	0.0
7	0.0
8	0.0
9	0.0
10	17.2
11	59.5
12	4.6
13	0.0
14	0.0
15	4.8
16	2.0
17	2.5
18	0.0
19	0.0
20	0.1
21	4.6
22	81.3
23	0.1
24	21.2
25	1.1

*\*Study ended on April 25, 2014*